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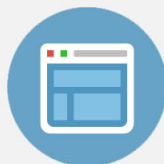
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Preparation of functional DNA microarrays through laser-induced forward transfer

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A functional DNA microarray was prepared through the laser-induced forward transfer (LIFT) technique. In a first experiment, droplets of a buffer solution were spotted onto a substrate at different laser pulse energies. This allowed one to determine that uniform spots with a diameter as small as 40 μm could be obtained. In a second experiment, a microarray containing two different human cDNAs and a negative control was spotted through LIFT and submitted to a hybridization assay. The obtained results demonstrated the full functionality of the microarray, which allowed us to prove the viability of LIFT for the production of DNA microarrays. © 2004 American Institute of Physics. [DOI: 10.1063/1.1787614]

The rise of DNA microarrays marked an important step in the development of sensors allowing parallel and rapid detection and identification of genes and gene mutations for genomics and biomedical diagnostic applications. The need for the production of low-cost microarrays with high spots density, precision, and repeatability has resulted in extensive research devoted to the design and development of different arraying techniques, the most extended ones being photolithography, pin deposition, and ink-jet printing.^{1,2}

Laser-induced forward transfer (LIFT) is a versatile non-contact technique that appears to be an interesting alternative to the conventional arraying techniques, since it avoids the use of expensive photolithographic processes and, thanks to the high focusing power of lasers, presents higher integration scales than pin deposition and ink-jet printing. Its method of operation consists in the focusing of a pulsed laser beam on a thin film of the material to be deposited through the film support transparent to the laser radiation. Under the action of the laser pulse, a tiny amount of the film material is transferred from the support to the flat substrate, which is placed parallel and at a short distance to the former one. Although LIFT was initially developed to deposit patterns of metals,³ its use was quickly extended to other inorganic materials⁴ and, more recently, to pastes and liquids.⁵

The possibility of transferring liquids through LIFT revealed that this technique could be applied to the deposition of organic and biological materials in solution, since the liquid solvent would act simultaneously as energy absorber and transport vector of the molecules. It was indeed demonstrated that in this way LIFT could be used to successfully transfer proteins^{6,7} and cells.^{6,8} There have also been attempts to use LIFT for the production of DNA arrays. Thus, Karaïskou *et al.* used a sub-picosecond laser to transfer double-stranded DNA directly from a DNA solid film, dem-

onstrating that undamaged DNA was indeed transferred in this way.⁹ In a different work we showed that double-stranded DNA could be transferred from a liquid film by means of a conventional nanosecond laser with good adhesion onto treated glass substrates.¹⁰ However, these results do not demonstrate the viability of LIFT for DNA microarray fabrication, since they do not grant that the amount of undamaged material adhered in each spot can provide enough signal intensity to be detected in a hybridization assay. In fact, a functional DNA microarray fabricated through LIFT allowing gene discrimination by means of conventional hybridization assays has not been reported yet.

In this work we present an operative DNA microarray fabricated through LIFT. After finding the conditions where regular and small DNA spots can be obtained, a microarray containing two different cDNAs and a negative control was spotted through LIFT and submitted to a hybridization assay with the perfect complementary strands of these cDNAs tagged with different fluorochromes. The detection of fluorescence intensity of a different wavelength for each cDNA and the absence of detected signal for the negative control would prove the viability of LIFT for the production of DNA microarrays.

Three different solutions were prepared for spotting: a buffer solution consisting in glycerol (50% in volume), dimethyl sulfoxide (25% in volume), and TrisEDTA (25% in volume), and two DNA solutions each one containing a single cDNA clone insert amplified by PCR. The buffer solution is a variation from a standard one normally used with conventional spotters, where glycerol was added in the right proportion to optimize the solution rheological properties for LIFT spotting. The two human cDNAs correspond to the mitogen-activated protein kinase 3 gene (MAPK3, 525 base pairs long, from the IMAGE clone 809939) the first one, and the v-ets avian erythroblastosis virus E26 oncogene homolog 2 (ETS2, 2205 base pairs long, from the IMAGE clone

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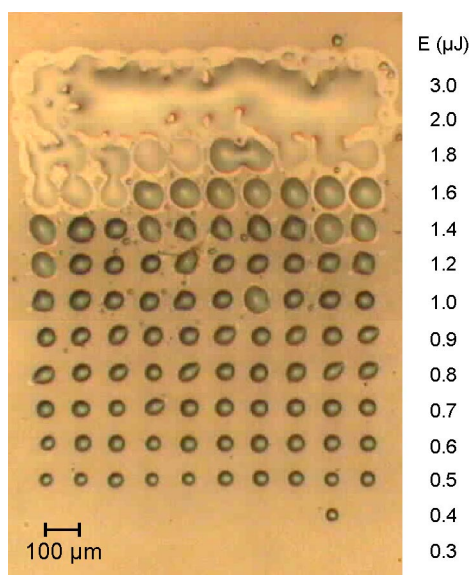


FIG. 1. (Color online). Optical microscopy image of the droplets obtained immediately after laser-induced forward transfer of a buffer solution film at different laser pulse energies.

260303) the second one, both in the previous buffer solution at a concentration of $250 \text{ ng}/\mu\text{l}$. A liquid film about $5 \mu\text{m}$ thick was prepared for each solution by spreading through a blade-coater a volume of $15 \mu\text{l}$ onto different Ti coated microscope slides. The Ti film (50 nm thick) acted as radiation absorber⁷ and the coated slide-liquid film system has been named ribbon.⁶ In the LIFT experiments the ribbons were placed parallel to the substrates where the material was going to be transferred at a distance of about $100 \mu\text{m}$, with the liquid film facing the substrate surface. The separation distance was kept through small kapton pieces and the receptor substrates were commercially available poly-*L*-lysine treated glass slides in all the cases.

LIFT was carried out by means of a pulsed Nd:YAG laser (355 nm wavelength, 10 ns pulse duration) whose nearly Gaussian beam was focused by a $15\times$ microscope objective onto the Ti absorbing film through the transparent rear side of the ribbon. The resulting beam diameter (full width at $1/e^2$ maximum) on the Ti film was $50 \mu\text{m}$. Transfer was carried out in such a way that each solution droplet in the array corresponded to a single laser pulse and the ribbon-substrate system was displaced with respect to the laser beam after each laser pulse by means of an *xyz* translation stage.

In a first experiment, a 10×14 array of the buffer solution was spotted through LIFT by varying through a diffractive variable attenuator the laser pulse energy between 0.3 and $3.0 \mu\text{J}$ in such way that each row in the array corresponded to a different laser pulse energy. An optical microscopy image of the obtained droplets array is presented in Fig. 1. Four different regions can be observed. At high laser pulse energies, between 1.6 and $3.0 \mu\text{J}$, the transferred droplets are so large that they coalesce with their adjacent neighbors. At laser pulse energies between 0.8 and $1.4 \mu\text{J}$ separated droplets are obtained, but they present irregular shapes, with a lot of satellites around them and considerable dispersion in size. In the third region, between 0.5 and $0.7 \mu\text{J}$, uniform, round-shaped droplets are obtained. Finally, below $0.5 \mu\text{J}$ no material was transferred to the substrate (the isolated droplet at

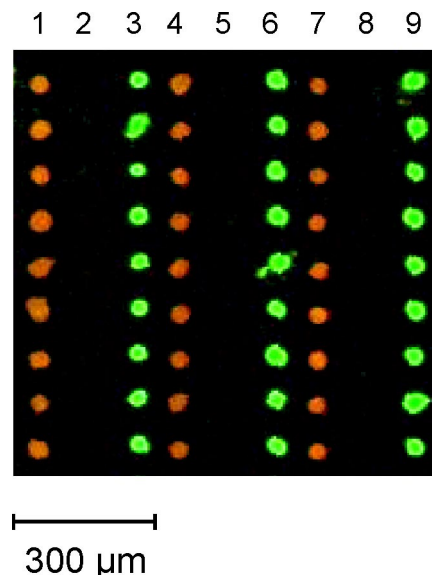


FIG. 2. (Color online). Fluorescence image obtained after hybridization of a microarray containing the two human cDNAs MAPK3 (columns 1, 4, 7) and ETS2 (columns 3, 6, 9) and the DNA-free solution (columns 2, 5, 8). The red emission in columns 1, 4, 7 corresponds to Cy5 tagged MAPK3 and the green emission in columns 3, 6, 9 to Cy3 tagged ETS2.

$0.4 \mu\text{J}$ corresponds to an extraordinarily intense random laser pulse). The droplets diameter decreases with energy and a minimum of about $40 \mu\text{m}$ has been obtained at $0.5 \mu\text{J}$. Therefore, it can be concluded that, among the explored conditions, $0.5 \mu\text{J}$ is the optimum laser pulse energy to obtain microarrays with the best uniformity and the highest integration scale.

In a second experiment, three different ribbons were used to spot through LIFT a 9×9 array at a laser pulse energy of $0.5 \mu\text{J}$. Each ribbon was prepared with one of the three different solutions described earlier and the array was spotted in alternative columns, each one containing droplets of a different solution. Therefore, the columns contained, in alternative order, the MAPK3 cDNA, the buffer solution alone and the ETS2 cDNA. Once prepared, the array was dried, and UV cross-linking of the transferred DNA was carried out in a Stratalinker at 130 mJ . Finally, the array was submitted to a standard hybridization protocol¹¹ with the complementary strands of the transferred DNA, each one tagged with a different fluorochrome: Cy5 for the MAPK3 cDNA and Cy3 for the ETS2 cDNA (Rediprime™ II Random Prime Labeling System from Amersham Biosciences). Once hybridized, the array was analyzed through a fluorescence scanner operating at 635 and 543 nm to excite Cy5 and Cy3, respectively (GenePix4000 from AxonInstruments Inc.). The recorded fluorescence image (Fig. 2) shows that the columns corresponding to the MAPK3 cDNA present the Cy5 red signal, the ones corresponding to the ETS2 cDNA present the Cy3 green signal and that there is no fluorescence signal in the ones with the negative control (buffer solution alone). This indicates that hybridization occurred only where DNA was deposited, with the required spatial resolution (about $40 \mu\text{m}$), and that the amount of DNA per spot was high enough to be easily detected with a conventional fluorescence scanner. Furthermore, the presence of a different

color for each column corresponding to a different cDNA shows that the microarray is indeed selective in DNA discrimination.

Summarizing, we can conclude that we have prepared a functional DNA microarray through LIFT. The analysis of the transfer process has allowed us to decrease the spot diameter to only 40 μm . In addition, it has to be pointed out that this spot diameter has not been optimized and that, by varying the laser focusing conditions and/or the thickness of the liquid film, smaller droplets could be obtained. The hybridization test has demonstrated that not only the array hybridizes with enough fluorescence intensity to be detected with a fluorescence scanner but also that this hybridization is really specific, which allows one to discriminate between different genes. Therefore, we can state that the viability of LIFT for DNA microarrays production has been effectively proven.

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